Chemical disinfection of combined sewer overflows

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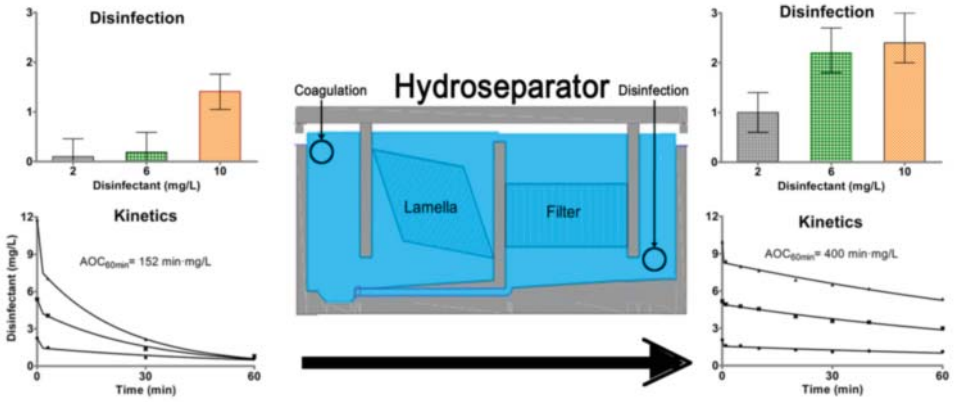

Ravi Kumar Chhetri

PhD Thesis
September 2017

DTU Environment
Department of Environmental Engineering

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Preface

The research contained within this PhD thesis was carried out at the Department of Environmental Engineering, at the Technical University of Denmark. It was conducted from October 2014 to September 2017 under main supervision of Professor Henrik Rasmus Andersen and co-supervision from Professor Hans-Jørgen Albrechtsen.

The thesis is organised in two parts: the first puts into context the findings of the PhD in an introductive review, while the second part consists of the papers listed below. These will be referred to in the text by their paper number, written in Roman numerals **I-X**.

I **Chhetri R.K.**, Thornberg, D., Berner, B., Gramstad, R., Öjstedt, U., Sharma, A.K., Andersen H.R.: Chemical disinfection of combined sewer overflow waters using performic acid or peracetic acids. *The Science of Total Environment 2014, 490, 1065-1072*

II **Chhetri R.K.**, Flagstad, R., Munch, E.S., Hørning, C., Berner, J., Kolte-Olsen, A., Thornberg, D., Andersen H.R.: Full-scale evaluation of combined sewer overflows disinfection using performic acid in a sea-outfall pipe. *Chemical Engineering Journal 2015*, 270, 133-139

III **Chhetri R.K.**, Bonnerup, A., Andersen H.R.: Combined Sewer Overflow pre-treatment with chemical coagulation and a particle settler for improved peracetic acid disinfection. *Journal of Industrial and Engineering Chemistry 2016*, 37, 372-379

IV **Chhetri R.K.**, Baun, A., Andersen H.R.: Algal toxicity of the alternative disinfectants performic acid (PFA), peracetic acid (PAA), chlorine dioxide (ClO_2) and their by-products hydrogen peroxide (H_2O_2) and chlorite (ClO_2^-). *International Journal of Hygiene and Environmental Health 2017*, 220, 570-574

V **Chhetri R.K.**, Klupsch, E., Jensen, P.E., Andersen H.R.: Treatment of arctic wastewater by Chemical Coagulation, UV and Peracetic acid disinfection. *Environmental Science and Pollution Research* DOI: 10.1007/s11356-017-8585-5

VI **Chhetri R.K.**, Baun, A., Andersen H.R.: Acute toxicity and risk evaluation of the CSO disinfectants performic acid, peracetic acid, chlorine dioxide and their by-products hydrogen peroxide and chlorite. *Water Research (in review)*

VII **Chhetri R.K.**, Kaarsholm, K.M.S., Andersen H.R.: Colorimetric quantification of peroxycarboxylic acid and hydrogen peroxide for water disinfection. *Submitted*

VIII **Chhetri R.K.**, Di Gaetano, S., Turolla, A., Albrechtsen, H-J., Antonelli, A., Andersen H.R.: Study of disinfection efficiency of peracetic acid (PAA) on *Escherichia coli* after eliminating hydrogen peroxide from the commercial PAA mixture. *Manuscript*

IX **Chhetri R.K.**, Di Gaetano, S., Turolla, A., Antonelli, A., Andersen H.R.: Synergic effect of peracetic acid and hydrogen peroxide on *Escherichia coli* disinfection. *Manuscript*

X **Chhetri R.K.**, Di Gaetano, S., Turolla, A., Antonelli, A., Andersen H.R.: Ecotoxicity evaluation of pure peracetic acid (PAA) after eliminating hydrogen peroxide from commercial PAA. *Manuscript*

I was a guest editor in the special issue of ***International Journal of Hygiene and Environmental Health***. In addition, the results of the thesis have also been presented at several conferences:

- **Chhetri R.K.**, Bonnerup A., Andersen H.R.: Chemical coagulation and disinfection of combined sewer overflow using poly-aluminium chloride and peracetic acid, Paper presented at CEST 2015- 14th International Conference on Environmental Science and Technology, Rhodes, Greece

- **Chhetri R.K.**, Klupsch E., Andersen H.R., Jensen P.E.: Wastewater treatment in Kangerlussuaq, Greenland by Chemical coagulation and UV disinfection, Paper presented at Artek 2016- International Conference on Sanitation in Cold Climate Regions, Sisimiut, Greenland

- **Chhetri R.K.**, Baun A., Andersen H.R.: Eco-toxicological screening of peracetic acid and performic acid for combined sewer overflow disinfection, Paper presented at Cannes 2016- 8th International Water and Health Seminar, Cannes, France

- **Chhetri R.K.**, Di Gaetano, S., Turolla, A., Antonelli, A., Andersen H.R.: Study of disinfection efficiency of peracetic acid (PAA) on *Escherichia coli* by rapid colorimetric assay based on enzymatic substrates after eliminating hydrogen peroxide from the commercial PAA mixture, Paper presented at CEST 2017- 15th International Conference on Environmental Science and Technology, Rhodes, Greece

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Foremost I would like to thank and acknowledge my supervisor Professor Henrik Rasmus Andersen. I feel grateful for having had the opportunity to work under such competent and friendly guidance. I have particularly enjoyed our meeting, starts in English and always ends in Danish, enabling me to keep up with the Danish language. I am also grateful for the support of my co-supervisor, Professor Hans-Jørgen Albrechtsen.

I would like to thank Dr. Kamilla MS Kaarsholm for friendly discussion in an inspiring atmosphere. A special thanks goes to Anne Harsting for supporting constantly during these three years. I particularly wish to thank Kai Tang, Aikaterini Spiliotopoulou, Dongah Ko, Ariadni Droumpali and Gordon Ooi for making my time here so special. I am very grateful to Silvia Di Gaetano for her assistance during her stay in Denmark. I owe a huge thank to Lene Kirstejn Jensen, Mona Refstrup, Sabrina Nedell Nissen, Charlotte Lind, Christina Pannach, Jens Scchaarup Sørensen, Sinh Hy Nguyen and Kim Ryberg for their help.

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Summary

In Copenhagen, a significant number of harbour bathing areas are occasionally closed for recreational activities, due to the discharge of untreated combined sewer overflows (CSOs). A CSO event occurs when the designed capacity of the combined sewer system is exceeded during major rainfall events. A CSO, a variable mixture of wastewater and rainwater, is discharged into the nearby surface water, which renders surface water unusable for recreational activities, such as bathing. This is because the microbial water quality of receiving waters is not of a suitable quality as mentioned in the EU directive 2006/7/EC. Nordic countries have a short summer season, and the frequent closures of harbour bathing areas in prime weeks for recreational activities are due mainly to the discharge of untreated CSO. Disinfecting a CSO in the existing CSO structure, before discharging it to the surface water, would be a quick way to maintain limits on the indicator bacteria of 500 MPN *Escherichia coli* (*E. coli)* per 100 mL and 200 MPN *Enterococcus spp* per 100 mL in the receiving waters. Disinfecting CSO has not been practiced before in Denmark, but it would increase the usability of surface waters for recreational activities. The occurrence of a CSO event, and its quality and quantity, is unpredictable, so the disinfectants employed for such a task should be robust, in order to treat water varying in quality.

The present thesis provides a solution to designing a CSO disinfection system, without changing CSO overflow structures. An overview of the chemical disinfection of a CSO, from the batch scale to the full-scale, was studied, and disinfection efficiency was evaluated by calculating the removal of bacteria from a CSO and quantifying disinfectants during treatment. Residual toxicity was studied for a preliminary risk assessment of disinfectants entering the aquatic ecosystem in the receiving water's post-disinfection discharge.

Performic acid (PFA) and peracetic acid (PAA) are used to disinfect CSO water, in order to reduce the number of indicator bacteria. Moreover, PFA and PAA do not form toxic by-products when they react with the ammonia present in the CSO. Disinfectant dose and contact time in the present study were designed by disinfecting a laboratory-simulated CSO with different wastewater concentrations. Degradation kinetics of PFA and PAA in the simulated CSO as well as the disinfection efficiency were studied. PAA degradation in the simulated CSO was slower compared to the degradation of PFA, the latter of which, at a dose (1-8 mg/L) and with 10 minutes' contact time, efficiently removed 4.2 logs of *E. coli* and 3 logs of *Enterococcus spp* from the simulated

CSO. Furthermore, the ecotoxicity of the residual disinfectants PFA, PAA and chlorine dioxide (ClO_2), and their degradation products hydrogen peroxide and chlorite, in relation to organisms in the aquatic ecosystem was studied. With the help of ecotoxicity data, a preliminary environmental risk assessment of PFA, PAA and ClO_2 for CSO disinfection was done, to ensure the safety of the aquatic ecosystem in the receiving waters. This assessment could also be used to obtain permission from authorities for full-scale disinfection. Based on the maximum allowable concentration quality standards for the freshwater and predicted residual concentrations of PFA, PAA and ClO_2, a minimum dilution factor (590 times for PFA, 138 times for PAA and 700 times for ClO_2) is needed for discharge into the surface water, to avoid the risk of toxic effect in the aquatic environment, albeit the rapid degradation of PFA and ClO_2 in water will not have an acute toxic effect, and lower dilution factors may also be safe for the receiving waters.

PFA and PAA were applied for the full-scale disinfection of CSO in two different Danish CSO structures. In the first CSO events, 2-8 mg/L PFA with 20 minutes' contact time efficiently reduced *E. coli* and *Enterococcus spp* below the limit mentioned in EU directive 2006/7/EC, when treated CSO was diluted into the Øresund strait. In the second CSO event, however, low PFA (1-4 mg/L) failed to reduce the number of *E. coli* and *Enterococcus spp* bacteria below the limit mentioned in the EU directive, even after dilution, entering the Øresund. PAA was used for full-scale disinfection when CSO was pretreated with chemical coagulation and through the HydroSeparator to remove suspended solids. During the CSO event, 10 mg/L PAA reduced *Enterococcus spp* from $10^{5.5}$ MPN per 100 mL to $10^{3.7}$ MPN per 100 ml with 10 minutes' contact time. Microbial profiles, made by measuring *Enterococcus spp* before and after a CSO event, revealed that the numbers of *Enterococcus spp* post-disinfection were almost the same as pre-existing *Enterococcus spp* in the first recipient. To summarise, frequent closures of recreational areas can be minimised by chemically disinfecting CSOs before discharging into surface waters.

Dansk sammenfatning

I København lukker et betydeligt antal havnebadeområder af og til for fritidsaktiviteter på grund af udledning af ubehandlede overløb fra fælleskloak (Combined sewer overflow-CSO). En CSO-begivenhed opstår, når det kombinerede kloaksystems design kapacitet overskrides ved store nedbørshændelser. En CSO, en variabel blanding af spildevand og regnvand, udledes i det nærliggende overfladevand, hvilket giver overfladevand uegnet til fritidsaktiviteter, såsom badning, da den mikrobielle vandkvalitet ikke lever op til kravene nævnt i EU-direktiv 2006/7/EC. Norden har en kort sommersæson, og de hyppige lukninger af havnebade i hovedsæsonen for vandrelateret fritidsaktiviteter skyldes primært udledning af ubehandlet CSO. Desinficering af CSO i den eksisterende CSO-struktur, inden den udledes til overfladevandet, ville være en hurtig måde at opretholde den mikrobielle vandkvalitet målt ved indikatorbakterierne *Escherichia coli* (*E. coli*) og *Enterokokker spp* med henholdsvis en grænse på 500 MPN *E. coli* pr. 100 ml og 200 MPN *Enterokokker spp* pr. 100 ml. Desinficering af CSO er ikke tidligere blevet praktiseret i Danmark, men det ville øge anvendeligheden af overfladevand til fritidsaktiviteter. Forekomsten af en CSO-begivenhed og dens kvalitet og mængde er uforudsigelig, så desinfektionsmidlet, der anvendes til en sådan opgave, bør være robust for at behandle vand, der varierer i kvalitet.

Den foreliggende afhandling giver en løsning på hvordan et CSO desinfektionssystem kan designes uden at ændre CSO overløbsstrukturer. Undersøgelser af kemisk desinfektion af CSO, fra batchskala til fuldskala, blev gennemført, og desinfektionseffektiviteten blev evalueret ved fjernelse af bakterier fra CSO samt kvantificering af desinfektionsmidler under behandlingen. Toksiciteten af det restende kemikalie blev undersøgt for en præliminær risikovurdering af desinfektionsmidler, der udledes i akvatiske økosystem i modtagervandets udledning efter desinfektion.

Permyresyre (PFA) og pereddikesyre (PAA) kan anvendes til at desinficere CSO-vand for at reducere antallet af indikatorbakterier da PFA og PAA ikke danner giftige biprodukter, når de reagerer med ammoniak der er tilstede i CSO. Desinfektionsdosis og kontakttid i den foreliggende undersøgelse blev designet ved at desinficere en laboratoriesimuleret CSO indeholdende forskellige spildevandskoncentrationer. Nedbrydningskinetik af PFA og PAA i den simulerede CSO og desinfektionseffektivitet blev undersøgt. PAA-nedbrydning i den simulerede CSO var langsommere sammenlignet med

nedbrydningen af PFA, hvoraf sidstnævnte i en dosis (1-8 mg/L) og med 10 minutters kontakttid fjernede 4,2 dekader af *E. coli* og 3 dekader af *Enterokokker spp*. Desuden blev økotoksiciteten af de resterende desinfektionsmidler PFA, PAA og klordioxid (ClO_2) og deres nedbrydningsprodukter hydrogenperoxid og chlorit undersøgt i forhold til organismer i akvatiske økosystemer. Ved hjælp af økotoksicitetsdata blev der foretaget en præliminær miljørisikovurdering af PFA, PAA og ClO_2 til CSO-desinfektion for at beskytte det akvatiske økosystem i recipienten. Denne vurdering kan også bruges til at få tilladelse fra myndighederne til fuldskala desinfektion. Baseret på de maksimalt tilladte koncentrationskvalitetsstandarder for ferskvand og forudsagte restkoncentrationer af PFA, PAA og ClO_2, skal en fortyndingsfaktor (590 gange for PFA, 138 gange for PAA og 700 gange for ClO_2) overholdes ved udledning i overfladevand, for at undgå risikoen for forgiftning af vandmiljøet, omend den hurtige nedbrydning af PFA og ClO_2 i vand gør at disse ikke vil have en akut toksisk virkning, og lavere fortyndingsfaktorer også kan være sikre for det vandmiljøet.

PFA og PAA blev anvendt til fuldskala desinfektion af CSO i to forskellige danske CSO strukturer. I de første CSO-hændelser reducerede 2-8 mg/L PFA med 20 minutters kontakttid effektivt *E. coli* og *Enterokokker spp* til under den grænse, der er nævnt i EU-direktiv 2006/7/EF, da CSO blev fortyndet i Øresund. I den anden CSO-hændelse, reducerede en lav PFA dose (1-4 mg/L) ikke antallet af *E. coli* og *Enterokokker spp* bakterier til under grænseværdien givet i EU-direktivet, selv ikke efter fortynding i Øresund. PAA blev anvendt til fuldskala desinfektion af CSO som var forbehandlet ved kemisk koagulation og en HydroSeparator for at fjerne suspenderet stof. Under CSO-begivenheden reducerede 10 mg/L PAA *Enterokokker spp* fra $10^{5,5}$ MPN pr. 100 ml til $10^{3,7}$ MPN pr. 100 ml med 10 minutters kontakttid. De målte mikrobielle profiler, som blev foretaget ved at måle *Enterokokker spp* før og efter en CSO-begivenhed, viste, at antallet af *Enterokokker spp* post-desinfektion var næsten det samme som allerede eksisterende *Enterokokker spp* i den første recipient. Sammenfattende kan de hyppige lukninger af rekreative områder minimeres ved kemisk desinfektion af CSO'er, før de udledes i overfladevand.

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Abbreviations

CSO	Combined Sewer Overflow
MPN	Most Probable Number
CFU	Colony-Forming Unit
SS	Suspended Solids
E. coli	*Escherichia coli*
PAA	Peracetic acid
PFA	Performic acid
ClO_2	Chlorine dioxide
H_2O_2	Hydrogen peroxide
PNEC	Predicted No-Effect Concentration
EQS	Environmental Quality Standards
MAC-QS	Maximum Allowable Concentration Quality Standards

1 Introduction

1.1 Background

Combined sewer systems, in which wastewater is mixed with rainwater and transported to a wastewater treatment plant for processing, are common in many cities. When major rainfall events occur, the designed capacity of combined sewer systems can be exceeded, which results in the discharge of untreated combined sewer overflows (CSOs) into nearby surface waters. Generally, CSO water is discharged to the receiving waters by either a long CSO discharge pipe or a long sea outfall pipe, or it can be retained in the retention basin (Figure 1). The discharge of untreated CSOs deteriorates the quality of receiving water, since it contains a varied mixture of rainwater, raw sewage, watershed run-off pollutants, variable pathogenic organisms, viruses, suspended solids, chemicals and floatable materials (USEPA, 1999). This results in the cessation of the usability of affected surface waters for bathing and other recreational activities, due to the risk of infections, and has knock-on economic effects, as many cities have redeveloped their harbours as recreational areas (Paper I). A red no-swimming flag is flown when the limit values of indicator organisms for good bathing water quality are exceeded. According to European Union directive 2006/7/EC (2006) for the good quality of bathing water, the number of indicator organisms should not exceed 500 MPN *Escherichia coli (E. coli)* per 100 mL and 200 MPN *Enterococcus spp* per 100 mL for water intended for recreational purposes. The Greater Copenhagen area has recently opened its harbours for recreational activities such as bathing. The DHI Group, in collaboration with different municipalities, informs citizens about bathing water quality on a number of beaches. Notifications are based on online data, obtained from wastewater treatment plants, on wastewater discharges, i.e. treated, by-passed or routed as overflows to receiving waters (www.badevand.dk). In the Greater Copenhagen area, 47 harbour bathing area were closed for recreational activities, due to the discharge of untreated CSOs (FRODO, 2014). As per data from 2006-2009, provided by HOFOR, the beach at Svanemøllebugten was closed for 14 days, and the recreational areas at Island Brygge and Fisketorvet were closed for 7 and 4.5 days, respectively, due to the discharge of untreated CSOs (FRODO, 2014). Denmark and other Nordic countries have a short summer season of around 3 months per year. However, the frequent closure of beaches and harbours in these prime recreational months, due to the discharge of untreated CSOs, could be minimised by disinfecting CSOs and thereby maintaining microbial bathing water quality in line

with European Union directive 2006/7/EC. Disinfecting CSOs has not been commonly practiced in Denmark, but it would help meet the limits for microbial loads jettisoned into receiving waters.

Figure 1: Schematic representation of a combined sewer overflow system.

1.2 Selection of disinfectants

There are various well-known disinfection technologies available that could be used to reduce contamination by microorganisms in CSO events, before their discharge into receiving waters, one of which is chemical disinfection. An ideal disinfectant should guarantee the maximum pathogenic microorganism removal efficiency, without generating toxic and undesirable by-products. In addition, it should be inexpensive and technologically compatible. The most common options are chlorine compounds, ozone and UV. Chlorine is less suitable for CSO disinfection, due to the formation of toxic, mutagenic and carcinogenic by-products (Bayo et al., 2009; Hrudey and Charrois, 2012; Watson et al., 2012; White, 2010). UV radiation, on the other hand, is an efficient disinfection method but it is expensive to install, while ozone is very efficient

also, but the high cost of the required generator and its installation make it unsuitable for disinfecting CSO events, which only occur a few times in a year. There are some important differences between disinfecting CSOs compared to wastewater, namely that CSO events only occur a few times per year, compared to the year-round continuous need for disinfecting wastewater. Furthermore, both the quality and the quantity of CSOs needed to be disinfected change rapidly during a CSO event, and therefore the process requires that the disinfectant is robust enough to change the quality of water and its dose can be changed rapidly, when required.

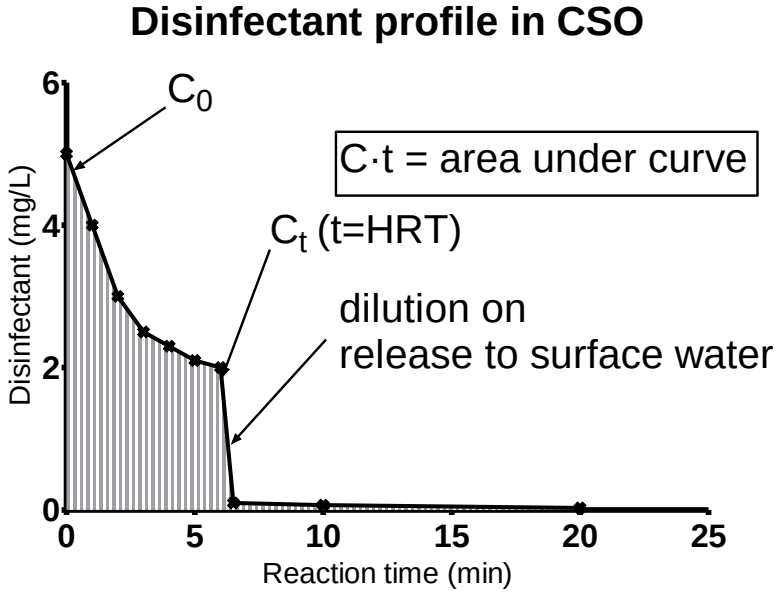

Figure 2: Design parameter of disinfectant for CSO disinfection

Disinfecting CSOs can be achieved in existing sewer systems by adding disinfectant at the beginning of the outlet pipe or before the detention tank. The amount of disinfectant to be added will depend on the quality of the CSO and the available residence time in the system. Before applying chemical disinfectant, it is necessary to investigate the degradation rates of chemicals in different types of CSO of variable quality. This is done by measuring the concentration (C) of disinfectant up to time (t), which is equivalent to the hydraulic retention time of the system. C·t is used to calculate the area under the curve (AUC), which is then correlated to the disinfection efficiency of targeted organisms. Ecotoxic effects of residual disinfectant on receiving waters and aquatic systems need to be investigated before discharge into receiving waters, as it helps calculate the dilution of disinfected CSO entering surface water (Figure 2).

Two organic peroxides, performic acid (PFA) and peracetic acid (PAA), emerge as alternatives to chlorine, since they do not react with the ammonia present in the CSO water to form chlorinated toxic by-products.

1.2.1 Performic acid

Performic acid (PFA) is a well-known disinfectant in medicine and the food industry (Gehr et al., 2009) and has been used to disinfect primary and secondary WWTP effluents in recent years (Gehr et al., 2009; Ragazzo et al., 2013) and combined sewer overflows (Paper I & II). PFA is an unstable product and needs to be generated on the spot, when needed, as a quaternary equilibrium mixture of PFA, formic acid, hydrogen peroxide and water:

$CH_2O_2+H_2O_2 \rightleftharpoons CH_2O_3+H_2O$ (Eq. 1)

PFA degrades into formic acid, which is not toxic to aquatic fauna and is readily biodegradable (Gehr et al., 2009; USEPA, 2001), and hydrogen peroxide, which is a weak disinfectant and degrades slower than organic peroxides (Wagner et al., 2002). Poffe et al. (1978) used hydrogen peroxide to disinfect municipal wastewater; however, it required a higher dose and a long contact time to achieve adequate disinfection. As hydrogen peroxide remains in the treated water, its toxicity to organisms in the receiving water needs consideration.

1.2.2 Peracetic acid

Peracetic acid was introduced to wastewater treatment around 30 years ago and is one of the most used disinfectants in wastewater treatment, due to its wide spectrum of antimicrobial activity (Antonelli et al., 2006; Baldry, 1983; Falsanisi et al., 2006; Kitis, 2004; Luukkonen et al., 2015). Recently, it has been used to disinfect CSOs and arctic wastewater (Paper I, III & V). Commercially available PAA is available as an acidic quaternary equilibrium mixture of peracetic acid (PAA), hydrogen peroxide, acetic acid and water:

$CH_3COOH+H_2O_2 \rightleftharpoons CH_3CO_3H+H_2O$ (Eq. 2)

Residues after PAA treatment use are acetic acid, hydrogen peroxide and water. Acetic acid is biodegraded further into carbon dioxide which is not toxic to aquatic life (Liberti and Notarnicola, 1999).

1.2.3 Chlorine dioxide

Chlorine dioxide (ClO_2) has been used as an alternative to chlorine, since it does not form chloramines and chlororganic compounds as toxic by-products (Hofmann et al., 1999). Moreover, ClO_2 has a higher oxidation capacity compared to chlorine, and it effectively inactivates microorganisms within a wide range of pH levels (Junli et al., 1997). ClO_2 is not storable and it needs to synthesise before application, though its stock solution is semi-storable. A stock solution of ClO_2 was synthesised by the reaction of chlorite with strong acid:

$$5ClO_2^- + 4H^+ \rightarrow 4ClO_2 + Cl^- + 2H_2O \quad \text{(Eq. 3A)}$$

During disinfection, ClO_2 is reduced to chlorite as a degradation product, as shown in Eq. 3B, by oxidising bacteria and other matter in the treated water, thus forming chlorite as a significant by-product of the treatment (Korn et al., 2002; Lee et al., 2004; Svecevicius et al., 2005):

$$ClO_2 + e^- \rightarrow ClO_2^- \quad \text{(Eq. 3B)}$$

1.3 Research objectives (aims)

The overall objective of the PhD project was to investigate a rapid solution for CSO treatment, in order to maintain the good microbial quality of surface waters when CSO is discharged. Chemically disinfecting CSO was investigated without changing existing overflow infrastructure. This project also investigated if disinfections can be used as a quick treatment of arctic wastewater, where conventional wastewater treatment plants pose several challenges.

The specific objectives of this PhD thesis were:

- To study the feasibility of applying chemical disinfectants when disinfecting CSOs **(Papers I, VIII & IX)**

- To develop a rapid colorimetric assay for disinfectant quantification, to control the disinfection process **(Paper VII)**

- To study the adverse environmental effects of residual disinfectants and their by-products post-CSO disinfection **(Papers IV, VI & X)**

- To study the disinfection efficiency of chemical disinfectants for full-scale application in existing CSO infrastructure **(Papers II, III & V)**.

2 Parameter design and optimisation of the disinfectants

2.1 Simulated CSO

The quality of a CSO varies over time, since the concentration of raw wastewater varies depending on the amount of rainfall. For the laboratory experiments, three CSO qualities were simulated by diluting freshly collected raw wastewater and mixing it with demineralised water, to match the matrix content of the overflow at different times in an overflow event. The CSO simulated with 40% wastewater represents the first flush of an overflow event, the CSO simulated with 15% wastewater represents typical overflow and the CSO simulated with 5% wastewater represents an extended overflow event with higher dilution (Passerat et al., 2011)(Paper I). The concentrations of raw wastewater used for simulated CSO water were based on ammonium concentrations in a time series of different CSO events from different locations in Denmark (Paper I). Chemical oxygen demand (COD), turbidity, conductivity and alkalinity matched the design data where these parameters were determined.

To prepare the simulated CSO, raw wastewater was collected from Lundtofte WWTP, Denmark, and stored at 4°C until the experiments were performed. Lundtofte WWTP has a catchment area of 32 km^2 and treats domestic wastewater for approximately 130,000 p.e. The simulated CSO was used for chemical and microbiological analysis before and after disinfection. Ammonia was measured in each collected wastewater sample, and in most of the experiments, COD, turbidity, conductivity and alkalinity were measured in both wastewater and the simulated CSO, to verify dilution and variability. Ammonia and COD in raw wastewater were 35.5 mg/L and 306 mg/L, respectively, and when diluted to prepare the simulated CSO with 40%, 15% and 5% wastewater, ammonia was 13.5, 4.9 and 1.75 mg/L and COD was 127, 26.5 and 8 mg/L, respectively (Paper I).

2.2 Quantification of disinfectants

The quantification of disinfectants is important in controlling the disinfection process. The measured concentration of disinfectant is used to calculate and correlate the removal of microorganisms from the disinfection procedure. PAA, PFA and hydrogen peroxide have been quantified previously, using DPD/iodide colorimetric assay (Bader et al., 1988; Falsanisi et al., 2006;

Pedersen et al., 2009) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) colorimetric assay (Pinkernell et al., 1997; Wagner et al., 2002) (Paper I). Wagner et al. (2002) and Gehr et al. (2009) used ABTS colorimetric assay to quantify PAA and PFA, by measuring the total concentration of peroxides and then subtracting the concentration of hydrogen peroxide. Pedersen et al. (2009) used DPD (N,N-dimethyl-p- phenylenediamine) colorimetric assay to measure peracetic acid and hydrogen peroxide separately, using different reaction conditions and combining peroxidase enzymes. In this project, a two-step method was developed for the simultaneous quantification of PAA, PFA and hydrogen peroxide present in the PAA/PFA mixture via ABTS colorimetric assay together with potassium titanium oxide oxalate (TiO-Ox). In the first step, 200 µL TiO-Ox was mixed with 2 mL PAA/PFA samples in a 1.0 cm polypropylene cuvette. In this step, hydrogen peroxide that is present in PAA/PFA reacts selectively with TiO-Ox to make a complex compound, which in this case was quantified at 400 nm. In the second step, a 350 µL PAA/PFA sample was mixed with 350 µL 1M acetic acid (pH 3.5) and 350 µL 1g/L ABTS in a 1.0 cm polypropylene cuvette. The colour was allowed to develop for 10 minutes in the case of PAA and 20 minutes in the case of PFA. In this step, PAA/PFA oxidised colourless ABTS to green coloured $ABTS^{\cdot+}$, which was quantified spectrophotometrically at 405 nm using a Varian Cary 200 UV-Vis photometer (Papers I & VII).

Chlorine dioxide was measured using the Hach Lange test kit LCK 310, and chlorite concentration was measured using ion chromatography coupled with an IonPac AS14 analytical column (4 mm × 250 mm, Dionex) and an IonPac G14 guard column (4 mm × 50 mm, Dionex). The eluent phase consisted of 8 mM Na_2CO_3 and 1 mM $NaHCO_3$. Chlorite was quantified by a Jasco 870-UV (Japan) UV-detector at λ = 340 nm (Paper IV).

2.3 Concentration profiles

Three types of simulated CSO were disinfected with different initial concentrations of PAA and PFA (1 –30 mg/L) and reaction times (5–360 mins), and the degradation of PAA and PFA over time was studied. Furthermore, the degradation of PAA and PFA in surface water, i.e. seawater and lake water, was also studied (Paper I).

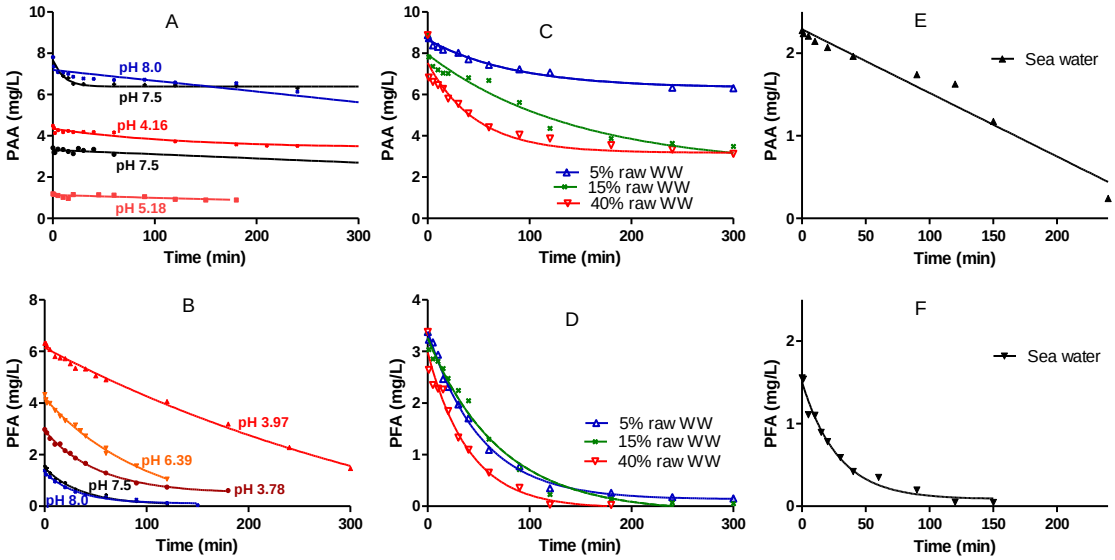

Figure 3: Concentration profiles of PAA (A) and PFA (B) at different pH levels in simulated extended overflow water (5% wastewater). Graphs C and D illustrate the matrix's influence on the concentration profile of organic peroxides. Graphs E and F show the concentration profiles of PAA and PFA in seawater collected near to the point the sea outfall pipe enters the Øresund (Paper I).

The degradation of PAA was slower than PFA in the simulated CSO with 5% wastewater (Figure 3A/B). The degradation of 6.32 mg/L PFA was roughly 36% after 120 minutes of contact time and 77% after 300 minutes, whilst degradation of 7.81 mg/L PAA was 22% after 300 minutes' contact time in the simulated CSO with 5% wastewater. In the experiment with 1.55 mg/L PFA in the simulated CSO with 5% wastewater, roughly 97% degraded after 120 minutes (Figure 3B). However, only 2% was degraded when 1.19 mg/L PAA was applied in the simulated CSO with 5% wastewater after 180 minutes (Figure 3A).

An initial consumption of PAA and PFA for all three types of simulated CSO water was increased when matrix density increased. In the simulated CSO with 15% wastewater (typical overflow), 11% of 8.45 mg/L PAA degraded within 1 minute, whilst 13% of 1.19 mg/L PAA was degraded for the same water

quality, also within 1 minute. In the first flush CSO (40% wastewater), 23% of 8.87 mg/L PAA was degraded after 1 minute. A similar effect on the initial consumption of PFA in the simulated CSO was observed. In typical overflow water, when 6.32 mg/L PFA was added, 10% degraded within 1 minute, and consequently 16 and 20% degradations were observed when 2.78 and 1.59 mg/L PFA were added, respectively, in simulated typical overflow water. This indicates that initial consumption increases when low concentrations of PFA are used. When 3.37 mg/L PFA was added in the extended overflow and first flush experiments, initial consumptions were approximately 5% and 22%, respectively, after 1 minute.

PAA degradation was stable in both acidic and neutral pH (Figure 3A). However, PFA degradation was stable in the acidic pH condition, whilst PFA degradation increased markedly at pH 7 and above (Figure 3B).

The degradation of PFA was faster than PAA in seawater (Figure 3E/F). PAA degraded faster in seawater compared to the simulated CSO water, but PFA degradation was similar to the simulated CSO water. When 2.2 mg/L PAA was added to the seawater, 91% degraded after 240 minutes, whilst 94% of 1.5 mg/L PFA degraded after 150 minutes for the same water quality.

2.4 Disinfection efficiency

In order to access disinfection efficiency, any residues of disinfectant in the samples were neutralised by adding sodium thiosulphate, to yield 100 mg/L, followed by catalase, to yield 50 mg/L (Wagner et al., 2002) (Paper I). *E. coli* was enumerated by the most probable number (MPN) method (APHA, 2012; 9308-2 ISO, 2012), using a 97-well Quanti-Tray and Colilert-18 reagent. *Enterococcus* was also enumerated using the MPN method with a 97-well Quanti-Tray and Enterolert reagent (APHA, 2012). The disinfection efficiency of PAA for *E. coli* in the extended overflow water was in the range of 3.4–5.6 log units when 2.5 to 30 mg/L PAA was added with 10 minutes' contact time (Figure 4A). However, the disinfection efficiency of PAA against *Enterococcus spp* was weak and the removal never exceeded 2 logs. PFA removed 1.8 to >5.7 logs of *E. coli* when 1 to 8 mg/L of PFA were applied with 10 minutes' contact time (Figure 4B). PFA always achieved a higher removal of indicator organisms with lower doses compared to PAA.

Based on the results obtained from PAA and PFA disinfection, *Enterococcus spp* was more difficult to remove with respect to *E. coli* (Figure 4A/B). There-

fore, disinfecting *Enterococcus spp* was considered in the following experiments, as it is certain that dimensioning disinfection systems, with the aim to maintain bathing water quality (Directive 2006/7/EC, 2006), has to be based only on *Enterococcus spp*.

Matrix density, and thus the number of indicator organisms present in CSO water, changes over time in a CSO event. To investigate its effect on the disinfection efficiency of PAA and PFA, experiments were carried out in the three types of simulated CSO water (Figure 4C/D). When 30 mg/L PAA was applied, 2 logs of *Enterococcus spp* were removed from simulated typical overflow water and first flush water. Except for the high PAA dose of 30 mg/L, the removal of *Enterococcus spp* failed to exceed 2 logs. When 4 to 12 mg/L of PFA was applied, more than 3 logs of *Enterococcus spp* were removed following 10 minutes' contact time. The disinfection efficiency of both PAA and PFA decreased slightly, in line with an increase in matrix concentration in the CSO (Figure 4C/D) (Paper I).

Experiments were carried out with 5 to 360 minutes of contact time, to investigate the effect of contact time on the disinfection efficiency of PAA and PFA (Figure 4E/F). A low PAA dose of 2.5 mg/L with 360 minutes of contact time removed more than 3 logs of *Enterococcus spp*, compared to 1.5 logs removed following 10 minutes of contact time. Similarly, 10 mg/L PAA with 240 minutes of contact time removed *Enterococcus spp* by more than 4 logs compared to 1.5 logs at 10 minutes' contact time. PAA was effective at removing *Enterococcus spp* with a long contact time, which makes PAA suitable for CSO structures that have a long retention time. The disinfection efficiency of PFA also increased in line with increased contact time, albeit on a much shorter time scale. The removal of *Enterococcus spp* was 3.8 logs with 4 mg/L PFA at 10 minutes' contact time, whilst the removal increased to >5 logs following 240 minutes of contact time. Applying 2 mg/L PFA with 10 minutes' contact reduced the number of *Enterococcus spp* by 3 logs, which makes PFA suitable for a treatment facility that has a short retention time.

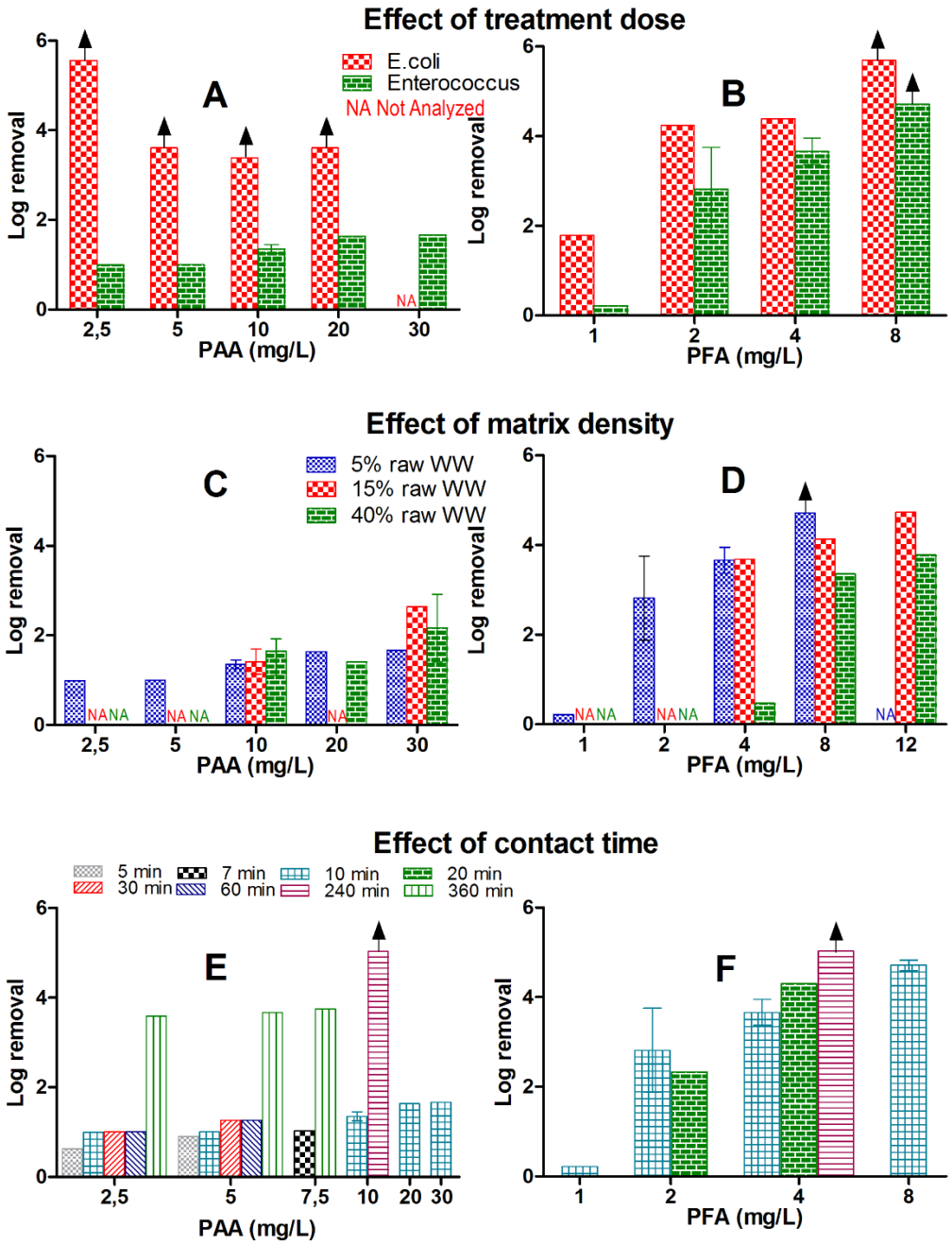

Figure 4: A/B) Disinfection efficiency of different initial concentrations of PAA and PFA on *E. coli* and *Enterococcus spp* in simulated extended overflow water (5% wastewater) with 10 minutes' contact time. C/D) Matrix influence on the disinfection efficiency of PAA and PFA with 10 minutes' contact time for *Enterococcus spp* on three simulated CSOs. E/F) The effect of initial concentrations of PAA and PFA and contact time on *Enterococcus spp* in simulated extended overflow water (5% wastewater). The T-bars indicate the range of the mean of the results when the treatment was repeated on different days (inter-day variability), while ▲ (arrow) indicates that the log removal is greater than the value shown by the bar, as the indicator organism MPN was below the detection limit after treatment (Paper I).

2.5 Preparation of pure PAA and disinfection

Commercial PAA is a quaternary equilibrium mixture of PAA, acetic acid and hydrogen peroxide. The degradation of hydrogen peroxide is slower than PAA (Wagner et al., 2002 & Paper I) and it has a stringent discharge limit into surface water. A study was conducted to investigate the disinfection efficiency of PAA_{alone} against *E. coli* when hydrogen peroxide was removed from the commercial PAA mixture. Moreover, the disinfection efficiency of PAA_{alone}, commercial PAA and hydrogen peroxide against *E. coli* was compared. Hydrogen peroxide from the commercial PAA was removed by titration, using potassium permanganate ($KMnO_4$) (Figure 5) (Paper VIII).

$$2MnO_4^- + 5H_2O_2 + 6H^+ \rightarrow 2Mn^{2+} + 5O_2 + 8H_2O \quad \text{(Eq. 4)}$$

In short, a working solution of 1 g/L PAA was titrated with 0.02 N potassium permanganate until the endpoint of a light-pink manganate appearance was achieved. Excess manganate was removed from the PAA by raising the pH of the solution to pH 8.5, where manganate crystals formed and were then filtered with a 0.45µm filter. To make the PAA solution stable, the pH of the PAA solution was maintained at 6.5, using phosphate buffer. The stability of PAA and the reappearance of hydrogen peroxide was monitored for 48 hours.

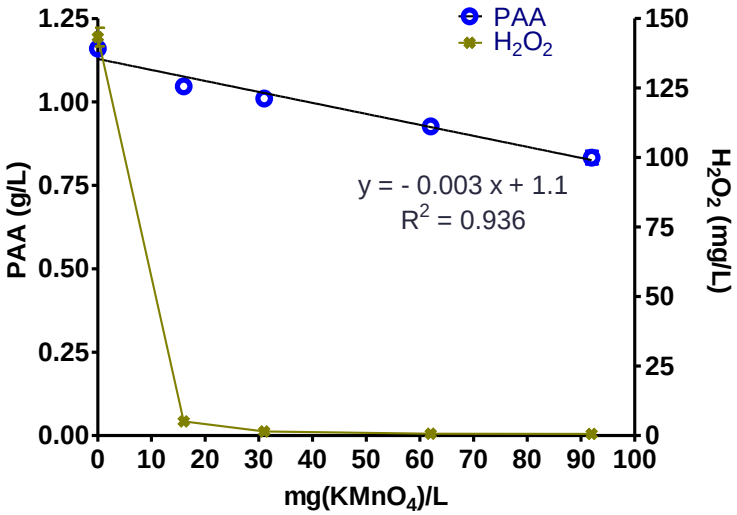

Figure 5: PAA and hydrogen peroxide concentrations measured at the end of titration with different concentrations of potassium permanganate (Paper VIII).

For the disinfection experiment, five concentrations of PAA_{alone} were added to the laboratory water, which was spiked with a known concentration of *E. coli*

(ATCC 25922). To study degradation, concentration profiles of PAA were observed for 60 minutes and an area under the curve (Ct) was determined. The residual PAA was neutralised by adding sodium thiosulphate, and the samples were processed for *E. coli* enumeration. The same experiments were repeated for commercial PAA and hydrogen peroxide. Median inhibition concentrations (IC_{50}) of PAA_{alone}, commercial PAA and hydrogen peroxide were estimated by using a nonlinear regression program, assuming lognormal distribution. By employing logistic curve fitting and inverse estimation inhibition concentrations (IC) were determined with corresponding 95% confidence limits.

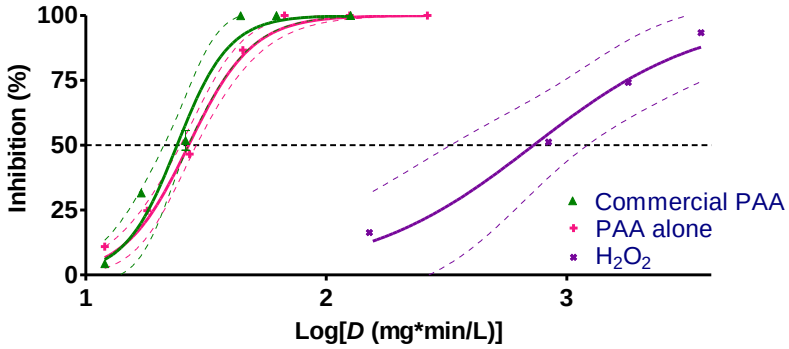

Figure 6: Inhibition-dose curves (thick lines) represented with their 95% confidence intervals (dashed lines). Observed fittings are characterised by the following coefficients of determination: commercial PAA, $R^2 = 0.99$; PAA_{alone}, $R^2 = 0.97$; H_2O_2 and $R^2 = 0.98$ (Paper VIII).

The disinfection efficiency of PAA_{alone}, commercial PAA and hydrogen peroxide was calculated by estimating the growth inhibition of *E. coli* exposed to disinfectants compared to the one without disinfectants. The median inhibition concentration (IC_{50}) of PAA_{alone} was 26.77 mg·min/L, whilst the IC_{50} of commercial PAA was 23.96 mg·min/L and the IC_{50} of hydrogen peroxide was 727.2 mg·min/L (Figure 6). The inhibition concentration of PAA_{alone} was higher than commercial PAA, which means that commercial PAA was more effective against *E. coli* than PAA_{alone}. A commercial PAA mixture has hydrogen peroxide in equilibrium, and hydrogen peroxide is a weak disinfectant. The IC_{50} of hydrogen peroxide was more than 27 times higher than PAA_{alone} and commercial PAA. Hydrogen peroxide is not effective against bacteria containing catalase enzymes, which convert hydrogen peroxide into water and oxygen. However, when commercial PAA was used to disinfect *E. coli*, it inactivated the catalase enzyme, thereby inhibiting hydroxyl radical oxidation (Kitis, 2004). Therefore, the difference in the IC_{50} of PAA_{alone} and commercial PAA was due to the synergic effect of hydrogen peroxide present in commercial PAA for

disinfection. The two-stage attack scheme of commercial PAA on bacteria was also explained by Flores et al. (2014), in which the synergic action of PAA and hydrogen peroxide was explained.

2.6 Disinfection mechanism

The mode of action of PAA as an antimicrobial agent by oxidising the sulfhydryl (-SH), disulphide (S-S) and double bonds in proteins and enzymes. Furthermore, PAA inactivates the catalase enzyme, which inhibits hydroxyl radical oxidation (Kitis, 2004). Denaturation of the protein, the bases of DNA molecules, the disruption of the chemiosmotic function of the lipoprotein cytoplasmic membrane and the inactivation of catalase enzymes are other mean disinfections offered by PAA. Moreover, PAA suppresses β-galactosidase activity (Lazarova et al., 1998) and bacteria are unable to recover from the damage caused by PAA and no re-growth takes place (Antonelli et al., 2006).

The synergic disinfection mechanism of PAA and hydrogen peroxide on *E. coli* was studied in two steps (Paper IX). First, half of the *E. coli* was inactivated for 60 minutes' contact time, using the median IC_{50} values of commercial PAA (0.95 mg/L) and PAA_{alone} (1 mg/L), and residual PAA was quenched by adding sodium thiosulphate. In this step, PAA partially damaged *E. coli* cells and inactivated catalase enzyme. In the second step, hydrogen peroxide was added as a disinfectant and a median IC_{50} value was calculated. The IC_{50} of hydrogen peroxide was reduced from 43.4 mg/L to 14.3 mg/L, when commercial PAA and PAA_{alone} was used to inactivate half of the *E. coli* (Paper XI).

In another experiment, the experimentally observed IC_{50} of commercial PAA and PAA alone was obtained by applying different nominal doses (C) of PAA and contact time (t). PAA degradation in a medium was considered when designing the IC_{50} values of PAA. The objective of this experiment was to check if the product of the variable nominal dose of PAA and contact time would result in the same inactivation of *E. coli* (50% inhibition). It was observed that a high PAA nominal concentration and short contact time have complete inactivation of *E. coli*, whilst less PAA nominal concentration with a long contact time had almost no effect on *E. coli* inactivation (Paper XI).

2.7 Ecotoxicity and indicative environmental risk assessment

An evaluation of the ecotoxic effect of residual disinfectants and their degradation products is important in accessing the potential impact of discharged disinfected effluents in receiving waters and aquatic systems. Moreover, ecotoxic data on disinfectants are important for obtaining permission from authorities to use them in full-scale applications, to ensure that disinfected effluents do not pose a toxic threat to the aquatic ecosystem. Generic aquatic risk assessments rely on laboratory-based tests carried out on organisms from different tropic levels in the ecosystem (ECHA, 2008). Ecotoxicity tests generally should cover degraders, primary producers and zooplankton in an aquatic ecosystem. Ecotoxicity tests used for this purpose are: the bacterial luminescence inhibition test with *Vibrio fischeri (V.fischeri)*, the crustacean immobilisation test with *Daphnia magna (D. magna)* and the algal growth inhibition test with freshwater green algae *Pseudokirchneriella subcapitata (P. subcapitata)*.

The toxicity of *V. fischeri* was measured with the commercial BioTox™ (AboatoxOy, Finland) assay kit. The tests were carried out in accordance with the ISO 11348-3 (2007) test method. Immobilisation tests with the crustacean *D. magna* were performed using the method and testing conditions prescribed by ISO 6341 (2012). Toxicity in relation to freshwater microalgae *P. subcapitata* was determined using modified ISO 8692 (2012). Effect concentrations (EC) with 95% confidence intervals for the inhibition of *V. fischeri* and *P. subcapitata* were estimated using a logistic regression (Logit) model. The ToxCalc™ v5.0 program was used to calculate the acute toxicity of *D. magna*. Lethal concentrations (LC) with 95% confidential intervals were calculated using the probit model along with linear regression by maximum-likelihood estimation (Tidepool Scientific).

Effect concentrations (EC_{10} and EC_{50}) and lethal concentrations (LC_{10} and LC_{50}) of the disinfectants and degradation products obtained from *V. fischeri, D. magna* and *P. subcapitata* toxicity are presented in Table 1 (Papers IV, VI & X).

Disinfectants (PFA, PAA, PAA_{alone} and ClO_2) were more toxic than their degradation products (hydrogen peroxide and chlorite), and among the three disinfectants, ClO_2 was the most toxic to *D. magna* and *P. subcapitata*, whereas this was not the case for *V. fischeri*. The toxicity (EC_{50}) of PAA was reduced for *V. fischeri* and *P. subcapitata* when hydrogen peroxide was removed from

a commercial PAA mixture; however, the LC_{50} of PAA and PAA_{alone} was similar for *D. magna* (Paper X). The reduction in toxicity on PAA_{alone} could have been due to the synergic effect of hydrogen peroxide presented in the commercial PAA. According to the CLP regulation, EC/LC_{50} values less than 1 mg/L are classified as "Acute toxic 1", i.e. very toxic to aquatic organisms (EU Commission, 2011), which means that PFA, PAA, PAA_{alone}, ClO_2 and chlorite are considered as very toxic for aquatic organisms, whereas this is not the case for H_2O_2.

Table 1: Effect concentration (EC_{10} and EC_{50}) for *V. fischeri* following 30 minutes' contact time, 72 hours algal growth rate inhibition tests with *P. subcapitata* and lethal concentration (LC_{10} and LC_{50}) for *D. magna* at 48 hours contact time of disinfectants (PFA, PAA, PAA_{alone} and ClO_2) and their degradation products (H_2O_2 and ClO_2^-). All concentrations are in mg/L and are based on nominal concentrations. 95% confidence intervals are shown in parenthesis (Papers IV, VI & X).

Organism	**Endpoint**	**Disinfectant chemicals**				**Degradation products**	
		PFA	**PAA**	**PAA_{alone}**	**ClO_2**	**H_2O_2**	**ClO_2^-**
Vibrio fischeri	EC_{10}	0.18 (0.13-0.23)	0.27 (0.26-0.27)	0.47 (0.38-0.58)	0.09 (0.04-0.24)	1.06 (0.52-2.16)	5.80 (3.63-9.25)
	EC_{50}	0.24 (0.21-0.27)	0.42 (0.41-0.44)	0.84 (0.78-0.91)	1.10 (0.66-1.84)	5.67 (4.20-7.65)	30.93 (24.96-38.33)
Daphnia magna	LC_{10}	0.59 (0.35-0.73)	0.53 (0.28-0.66)	0.45 (0.20-0.59)	<0.02	2.59 (1.79-3.01)	0.07 (0.01-0.18)
	LC_{50}	0.85 (0.67-0.98)	0.78 (0.59-0.95)	0.74 (0.55-0.91)	<0.09	3.46 (2.97-3.96)	0.36 (0.11-0.72)
P. subcapitata	EC_{10}	0.19 (0.12-0.32)	0.23 (0.10-0.53)	2.14	0.06 (0.05-0.07)	1.78 (1.63-1.94)	0.59 (0.59-0.60)
	EC_{50}	0.34 (0.29-0.39)	1.38 (0.96-1.99)	2.46	0.16 (0.15-0.17)	2.90 (2.87-2.92)	1.10 (1.10-1.11)

Concentration profiles were obtained by measuring the concentrations of disinfectants and degradation products over time in the media used for the toxicity analysis of *D. magna* and *P. subcapitata* (Paper IV & VI). Concentrations of disinfectants and degradation products were measured in samples at the same time point, for which the mortality of *D. magna* and the biomass quantification of *P. subcapitata* were recorded. According to OECD (2000), static ecotoxicity tests must be carried out under stable exposure conditions, i.e. where concentrations are maintained within 80-120% of the nominal concentration throughout the test period. However, this was not found in the literature data (Antonelli et al., 2009; Drábková et al., 2007; ECETOC, 2001; Liu et al., 2015; Mattei et al., 2006; van Wijk et al., 1998). In the case for PFA and ClO_2, papers IV & VI constitute the only data available for ecotoxicological evaluation.

Estimation of Predicted No Effect Concentration (PNEC) is the starting point for the indicative environmental risk assessment of disinfectants and their degradation products for CSO disinfection. $PNEC_{freshwater}$ values of disinfectants and their degradation products were calculated by dividing the lowest EC_{50} or LC_{50} value by assessment factors selected in accordance with the Technical Guidance Document (TGD-EQS, 2011). An assessment factor of 1000 was selected for all compounds, since data were available from short-term toxicity tests only at three trophic levels. The lowest EC/LC_{50} values of the respective compounds were divided by the assessment factor to calculate PNEC values (Table 2 & paper VI). PNEC values can be used as a basis for setting environmental quality standards (EQSs) with additional considerations of the potential for bioaccumulation and the persistency of the compounds (TGD-EQS, 2011). PFA, PAA and ClO_2 have no potential for bioaccumulation, as the $logK_{ow}$ values are far below 3 for all three compounds. Hence, the risk of secondary poisoning of predators in the aquatic ecosystem is very low. The fast degradation of PFA, PAA, ClO_2 and H_2O_2 in the test medium showed these compounds are not expected to be persistent in the aquatic environment.

Disinfecting a CSO occurs when a CSO event occurs, so continuous exposure of disinfectants and their degradation products is not expected to happen in the receiving water body. The environmental quality standard of relevance is the maximum allowable concentration for the freshwater ecosystem ($MAC\text{-}QS_{fw,eco}$) (TGD-EQS, 2011). To derive the $MAC\text{-}QS_{fw,eco}$, the same dataset with the lower assessment factor can be used, since intermittent discharges are mainly believed to result in acute effects. In this case, an assessment factor of 100 was chosen, in accordance with the TGD-EQS (2011), and thus the $MAC\text{-}QS_{fw,eco}$ for all compounds was ten times higher than the PNEC (Table 2 & paper VI).

When disinfected effluent is discharged into receiving waters, residual disinfectants and their degradation products must be maintained lower than $MAC\text{-}QS_{fw,eco}$, to avoid acute toxicity. This can be done either by destroying the residual disinfectants or by diluting the residual disinfectants. The dilution factors were calculated as the ratio between the predicted residual concentration (PRC) and the $MAC\text{-}QS_{fw,eco}$ of disinfectants and their degradation product, to avoid toxic effects in receiving waters.

Table 2: Predicted No-Effect Concentrations (PNECs), Environmental Quality Standards for water (EQS_{water}) and Maximum Allowable Concentration Quality Standards for the freshwater ecosystem ($MAC\text{-}QS_{fw,\ eco}$) for PFA, PAA, ClO_2, H_2O_2 and ClO_2^-. The individual dilution factors needed for an intermittent discharge not to be safe for a freshwater environment are the ratio between PEC and $MAC\text{-}QS_{fw,\ eco}$. All units are in µg/L (Paper VI).

	Disinfectant chemicals			**Degradation products**	
	PFA	**PAA**	**ClO_2**	**H_2O_2**	**ClO_2^-**
Predicted No-Effect Concentrations, $PNEC_{freshwater}$	0.24	0.42	0.09	2.9	1.1
EQS_{water}	0.24	0.42	0.09	2.9	1.1
$MAC\text{-}QS_{fw,\ eco}$	2.4	4.2	0.9	29	11
Predicted Residual Concentration, PRC*	170-1430	880	40-630	N.A	N.A
Dilution factor (PRC/MAC-QS)	70-590	138	44-700	N.A	N.A

N.A= Not available

*The Predicted Residual Concentration (PRC) is the concentration of disinfectants after treating a combined sewer overflow event (from Paper II & DesiCSO, 2014).

The residual concentrations of PFA, PAA and ClO_2 were higher than the $MAC\text{-}QS_{fw,eco}$ values for PFA and PAA. Therefore, maximum dilutions of 590 times of PFA, 138 times for PAA and 700 times for ClO_2 were needed, in order to avoid the risk of toxic effects in the aquatic environment.

PFA and PAA formulations have varying PFA/PAA:H_2O_2 ratios along with varying toxicity values. Hydrogen peroxide is also diluted when PFA and PAA are discharged and diluted into receiving waters post-disinfection. Rapid degradation of PFA (paper VI), in practice, will mean that a lower dilution factor than 590 may also be safe for receiving waters. Given the dilution factors shown in Table 2, it can be concluded that even though acute effects may occur at the initial point of discharge, due to the use of PFA and PAA as disinfectants, the relatively small dilution factors indicate that toxic effects will not occur after the initial dilution. ClO_2 showed the highest need for dilution; however, it has a relatively short half-life in water. This degradation leads to the formation of chlorite, but given the quite high MAC-EQ for chlorite (11 µg/L), this degradation product is not expected to lead to acute toxic effects in receiving waters after CSO disinfection with the mother compound (ClO_2) (Paper VI).

Overall, a preliminary environmental risk evaluation of disinfectants and their degradation was undertaken, to ensure environmental safety from the discharge of disinfected effluents post-disinfection. Furthermore, the ecotoxicity data

presented in this study complement the literature data on the ecotoxicity of alternative disinfectants and their degradation products.

3 Applied disinfection with PFA & PAA

3.1 PFA for the full-scale disinfection of CSO

A full-scale evaluation of CSO disinfection was performed using PFA in a sea outfall pipe in Skovshoved, a large wastewater pumping station north of Copenhagen (Paper II). In normal circumstances, the Skovshoved pump station relays wastewater from Gentofte municipality and part of the Lyngby municipality to the Lynetten wastewater treatment plant. During rain events, when pumping capacity is exceeded, CSO water bypasses the facility via a 1.6 km-long outfall pipe into the Øresund. The hydraulic retention time of CSO in the pipe is 24 minutes prior to discharge. For the full-scale disinfection of CSO water bypassed into the sea, PFA was generated onsite from the Disinfix unit (provided by Kemira Water, Denmark) and dosed to an overflow point in the existing CSO structure. Two autosamplers were installed to collect fractions of water, before and after PFA dosing, to determine the effects of treatment. It was not possible to collect disinfected water from the end of the sea outfall pipe to evaluate the disinfection effect, so a tube reactor made from flexible polythene pipe (1.55cm diameter and 100 m long) was installed to mimic the retention time in a sea outfall pipe. Each autosampler was set to collect 1L sample time proportionally every 20 minutes and up to 24 samples. The first sample collected in autosampler 1 corresponded to the second sample collected in autosampler 2, since there was a 20-minute retention time between the autosamplers. The activation of autosamplers and the initiation of PFA synthesis and dosing were automated based on signals from the digital control system of pumping station and the CSO system. To confirm the PFA dose delivered in the field, comparable PFA treatments were carried out on the three representative field-collected untreated samples from autosampler 1 in the laboratory. In order to match the onsite disinfection doses the in low and maximal flows of the CSO, three PFA doses were applied to each fraction. A fraction of each sample was processed for *E. coli* and *Enterococcus spp* enumeration after 20 minutes' contact time and in parallel concentration profiles of PFA was measured until 2 h in the remaining sample. From the first CSO event (28th Oct 2013), 16 samples (CSO fraction) were collected from autosampler 1 (non-disinfected) and 17 samples were collected from autosampler 2 (disinfected). Then, from the second CSO event (7th May 2014), 16 non-disinfected samples were collected but only 12 disinfected samples were collected, due to repeated clogging of the flexible polyethylene pipe collecting disinfected samples. All samples were analysed for conductivity, NH_4^+ and pH, as illustrated in Figure

7. The conductivity and NH_4^+ of the non-disinfected and onsite disinfected samples from both CSO events showed variations in CSO composition over time (Paper II). The conductivity and NH_4^+ increased during the first hour and decreased to reach a minimum during the second hour, remaining constant in both events. At the end of both CSO events, conductivity increased progressively, a finding which was also observed by Passerat et al. (2011). The pH of non-disinfected and disinfected samples showed some variation, but no trend was observed in either event, due to the variation in CSO composition over time. This shows that the acidity in the PFA mixture is insignificant, compared to the alkalinity in the water and its variation. The matching trend in the parameters between the influent and effluent of the disinfection system proves that the retention time of 20 minutes is correct (Paper II).

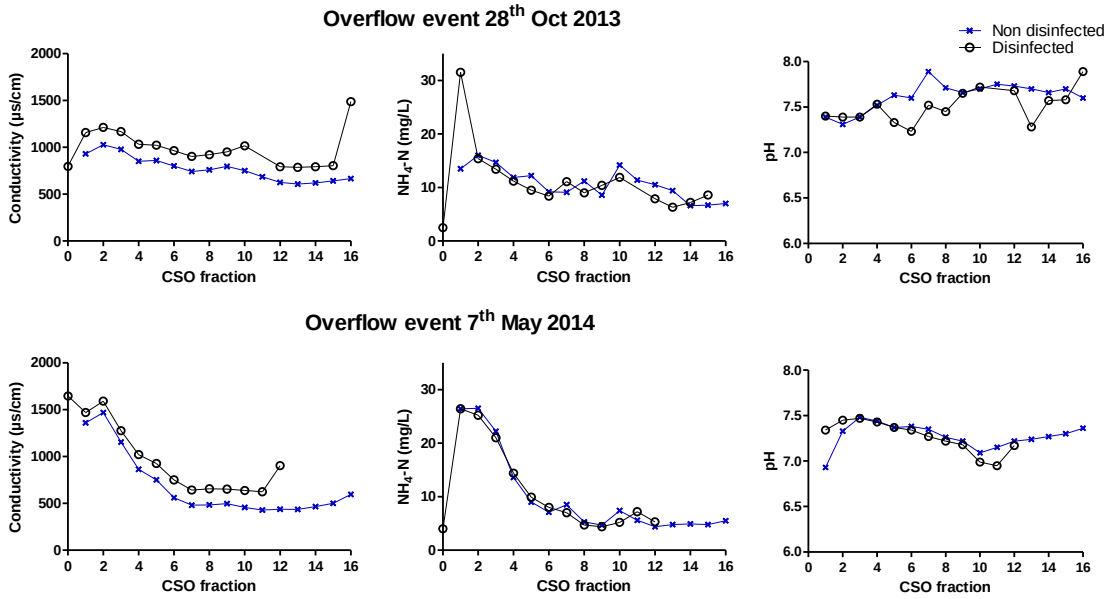

Figure 7: Conductivity, ammonium and pH of non-disinfected and disinfected CSO fractions from the CSO event occurring on 28th October 2013 (top) and 7th May 2014 (bottom) (Paper II).

The maximum initial start concentrations of *E. coli* and *Enterococcus spp* were $10^{6.3\text{-}5.6}$ and $10^{5.9\text{-}5.0}$ MPN, and the minimum were $10^{5.6}$ and $10^{5.0}$ MPN per 100 mL, respectively, in the first CSO event. Furthermore, in the first CSO event, the DesinFix unit was set to deliver a minimum 2 mg/L PFA, which later changed to variable CSO flow in the sea outfall pipe. Due to the failure in the automated starting of the DesinFix unit, the first three CSO fractions collected were not disinfected (Figure 8, Paper II). The removal of *E. coli* was from 2.5 to 3.0 log units and the removal of *Enterococcus spp* was from 1.0 to 2.3 log

units when the CSO fractions 4-8 were treated with 2.5-8 mg/L in the first CSO event.

In the second CSO event, the maximum initial start concentrations of *E. coli* and *Enterococcus spp* were $10^{6.9}$ and $10^{6.0}$ MPN, and the minimum were $10^{5.9}$ and $10^{5.2}$ MPN per 100 mL, respectively. A PFA dosing unit was set to deliver half the dose that was used in the first event. *E. coli* removal was 1.0 to 3.0 log units and *Enterococcus spp* removal was 1.0 to 2.44 log units when the CSO fraction was treated with 1-4 mg/L PFA. The removal of *E. coli* and *Enterococcus spp* decreased at the end of the overflow event, which was believed due to the clogging of the inlet to the tube reactor. The average *E. coli* and *Enterococcus spp* concentrations after full-scale disinfection were $10^{3.1}$ MPN *E. coli* and $10^{3.6}$ MPN *Enterococcus spp* per 100 mL of the CSO fraction in the first CSO event and $10^{4.9}$ MPN *E. coli* and $10^{4.8}$ MPN *Enterococcus spp* per 100 mL of the CSO fraction in the second CSO event. Considering the 75-fold dilution into the surface water, *E. coli* and *Enterococcus spp* concentration were below the limits mentioned in the EU directive (Directive 2006/7/EC, 2006) in the first CSO event. In the second CSO event, *E. coli* and *Enterococcus spp* concentrations were higher than the limit mentioned in the EU directive, even after 75-fold dilution in the surface water.

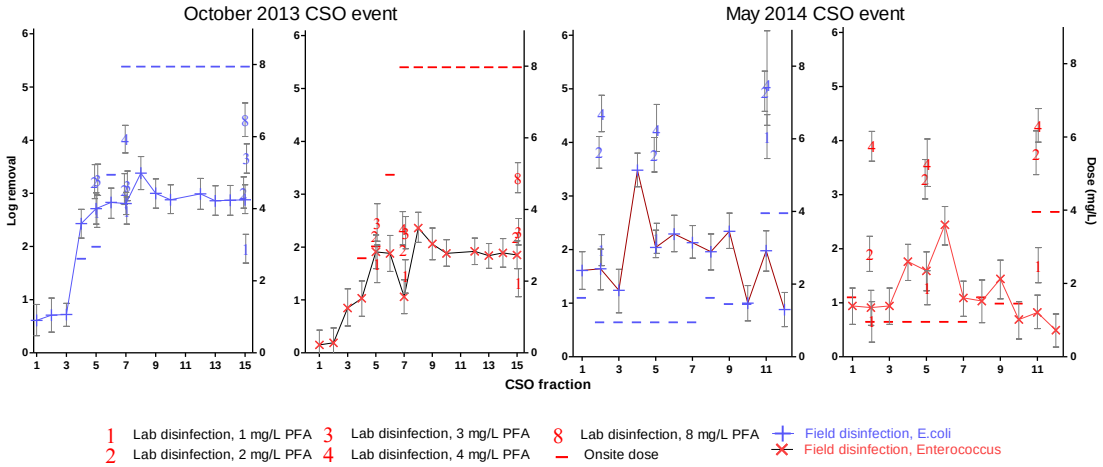

Figure 8: Dose (minus sign, right axis) and disinfection effects in the laboratory and field (symbols, left axis) of PFA on *E. coli* and *Enterococcus* in two CSO events from 28th October 2013 (two graphs from left) and 7th May 2014 (two graph from right). T-bars indicate the 95% confidence interval of experimentally observed disinfection effectiveness (Paper II).

Three selected fractions from each of the CSO events were disinfected with three (or four) different concentrations of PFA in the laboratory, to verify the

onsite disinfection of *E. coli* and *Enterococcus spp* (Figure 8 & Paper II). Furthermore, the degradation of PFA in the CSO fraction was observed up to 120 minutes. It was slow in the sample from the first CSO event, with residual PFA of 0.5-2 mg/L from 2.7, 3.7 and 7.5 mg/L PFA remaining after 120 minutes. In the second CSO event, complete degradation was observed after 120 minutes.

The removal of *Enterococcus spp* was lower than the removal of *E. coli* from the laboratory disinfection, which was also evident in Paper I and Ragazzo et al. (2013). Similarities in relation to the removal of *E. coli* and *Enterococcus spp* from the laboratory, and onsite disinfections were observed when low PFA doses were applied. However, when high doses of PFA were applied, the removal of *E. coli* and *Enterococcus spp* was higher in the laboratory disinfection. This dissimilarity was expected, though, as laboratory experiments are conducted in controlled conditions whereas the onsite disinfection of a real CSO event occurs under highly variable operating conditions and is affected by numerous factors (Paper II).

3.2 PAA for the full-scale disinfection of CSO

PAA was used for the full-scale disinfection of CSO water at the Kærby plant, located in the Middelfart city in Denmark, in order to treat the CSO from the towns Båring and Asperup during significant rain events. Kærby plant is equipped with the HydroSeparator® System (HydroSeparator), which is a patented and specialised system designed to treat CSO. It is equipped with lamella, followed by a mess filter with a sieve size of 20 microns (Figure 9). The principle use of the HydroSeparator is to remove suspended solids (SS) from CSO discharge during extreme weather events, which also, in turn, reduces pollutants associated with them. Particles greater than 4 mm present in the CSO water are removed by the mechanical screen prior flowing into the HydroSeparator, and the retention time of CSO water in the HydroSeparator is 7 minutes in maximal flow (25 L/s) conditions, whilst during minimum flow (5 L/s) the retention time is 35 minutes. Chemical coagulation by polyaluminum chloride (PAX XL 100) is employed at the inlet of the HydroSeparator, to optimise the removal of larger suspended solids. At the outlet, CSO water is disinfected with PAA in a reaction chamber, which then flows out onto constructed wetland.

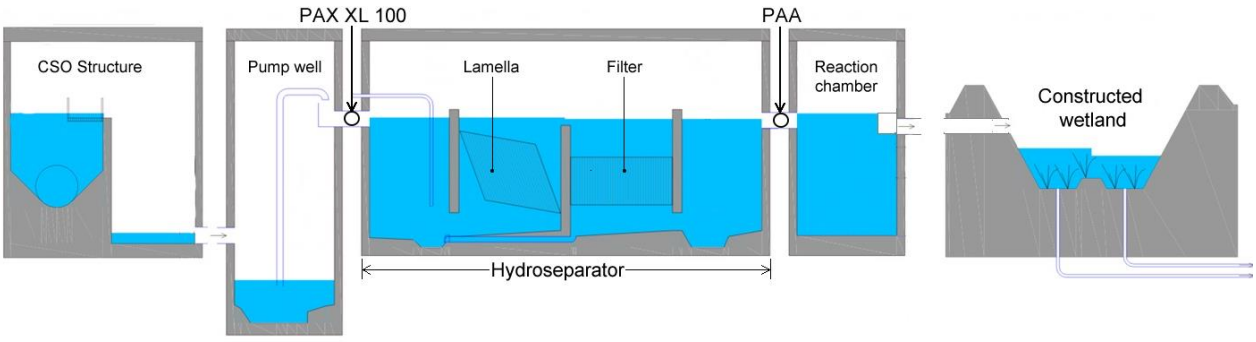

Figure 9: Schematic diagram of the Kærby plant, including the CSO overflow structure with a weir, the PAX dosing point before the HydroSeparator followed by a dosing point for PAA and a reaction tank finally leading to constructed wetland (Paper III).

The first two experiments were performed in the laboratory, in order to create the ideal dose of coagulant and disinfectant, whilst the final experiment was performed in the field, using the HydroSeparator system, dosing coagulant and disinfectant on a full scale.

To optimise the inflow of CSO into the HydroSeparator, three flow rates of 5, 15 and 25 L/s were tested, with the flow at 5 L/s efficiently removing suspended solids. A dose of chemical coagulant, PAX XL, was optimised by a traditional jar test in samples collected from the inlet of the HydroSeparator. Turbidity was reduced to 3 NTU from 52 NTU by applying 5 mg-Al/L with 15 minutes' sedimentation time. Similarly, phosphate was reduced to 0.63 mg/L from 2.43 mg/L by applying 5 mg-Al/L PAX XL 100 (Paper III).

The PAA dose for the full-scale experiment was optimised in the laboratory by disinfecting samples collected from the inlet and outlet samples with three different HydroSeparator flow rates. Three PAA concentrations of 2, 6 and 10 mg/L were added to the different samples, to study the disinfection efficiency and degradation of PAA for 180 minutes.

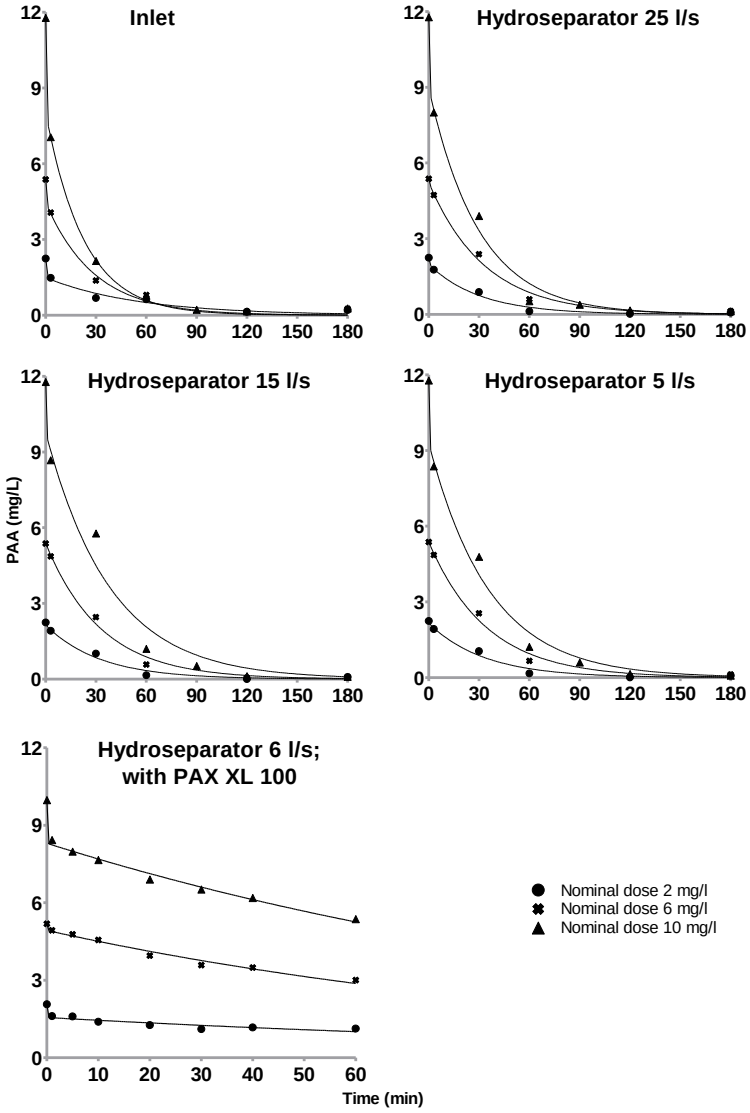

Figure 10: Concentration profile of different levels of PAA added to CSO water collected from the HydroSeparator with different flows and coagulant usage. The curve was fitted using modified first-order degradation kinetics via equation 5 (Paper III).

The initial consumption of PAA was high in all samples (Figure 10) and increased in line with the increased nominal PAA dose. Average initial consumption of PAA was 2.7 mg/L when 10 mg/L PAA was used in samples from the inlet and outlet of the HydroSeparator with different flow rates. Initial consumption was observed as 1.67 mg/L by applying the same concentration of PAA to disinfect the CSO water pretreated with the HydroSeparator and chemical coagulation. First-order degradation kinetics did not fit the observed data, due to a considerable initial consumption of oxidants. To address this initial consumption of PAA, Antonelli et al. (2006) and Falsanisi et al. (2006) applied a modified first-order kinetics expression to model the concentration profile of

PAA in wastewater effluents, including a parameter describing initial oxidant consumption as described by Haas and Finch (2001):

$$C_t = (C_0 - C_{Initial}) * e^{-kt} \qquad \text{(Eq. 5)}$$

In eq. 5, C_t is the residual disinfectant concentration at time t, C_0 is the applied disinfectant dose, $C_{Initial}$ is initial oxidant consumption, k is the rate constant and t is time. Fitted curves generated using this modified first-order expression are shown in Figure 10, with derived parameters presented in Table 3. The initial loss of PAA was neither observed in Paper I when it was used to disinfect CSO water from another site nor in primary treated wastewater (Wagner et al., 2002). High initial consumption and faster degradation of PAA might be due to the reaction of PAA with organic matter presented in CSO water.

Table 3: Fitted parameters of concentration curves and area under the curve for concentration profiles shown in Figure 8 (Paper III).

Sample	Nominal dose ($mg \cdot L^{-1}$)	$C_{Initial}$ ($mg \cdot L^{-1}$)	k (min^{-1})	R^2	AUC_{60min} ($mg \cdot L^{-1} \cdot min$)	AUC_{10min} ($mg \cdot L^{-1} \cdot min$)
Inlet	2	0.76	$1.8 \cdot 10^{-2}$	0.98	54	N.A
	6	0.92	$3.5 \cdot 10^{-2}$	0.99	120	N.A
	10	3.75	$4.4 \cdot 10^{-2}$	0.99	152	N.A
HydroSeparator 25 $L \cdot s^{-1}$	2	0.26	$3.2 \cdot 10^{-2}$	0.98	57	N.A
	6	0.14	$3.0 \cdot 10^{-2}$	0.99	156	N.A
	10	2.80	$3.3 \cdot 10^{-2}$	0.99	257	N.A
HydroSeparator 15 $L \cdot s^{-1}$	2	0.11	$3.1 \cdot 10^{-2}$	0.99	63	N.A
	6	~ 0	$3.0 \cdot 10^{-2}$	0.99	160	N.A
	10	1.99	$2.6 \cdot 10^{-2}$	0.98	330	N.A
HydroSeparator 5 $L \cdot s^{-1}$	2	0.09	$3.0 \cdot 10^{-2}$	0.98	65	N.A
	6	~ 0	$2.9 \cdot 10^{-2}$	0.99	164	N.A
	10	2.47	$2.8 \cdot 10^{-2}$	0.99	298	N.A
HydroSeparator 6 $L \cdot s^{-1}$; 5 mg-Al$\cdot L^{-1}$	2	0.51	$0.7 \cdot 10^{-2}$	0.92	75	16
	6	0.26	$0.9 \cdot 10^{-2}$	0.98	228	48
	10	1.67	$0.7 \cdot 10^{-2}$	0.99	400	81

N.A=Not analysed

Enterococcus spp concentration was reduced from $10^{5.0}$ MPN/100 mL to $10^{1.5\text{-}3.7}$ MPN/100 mL when 10 mg/L PAA was used to disinfect the samples obtained from the HydroSeparator with different flow rates. The reduction of *Enterococcus spp* was less in the inlet samples, which was explained by the more rapid degradation of PAA (Figure 10). Moreover, AUC_{60} of 10 mg/L PAA in the inlet sample was 152 mg·min/L, which was close to the AUC_{60} of 6 mg/L PAA (156-164 mg·min/L) for the HydroSeparator treated sample. Removal of *Enterococcus spp* from the full-scale disinfection of CSO post-treatment through the HydroSeparator and coagulation was 1.9 and 2.4 log units, after 10

minutes and 60 minutes' contact time, respectively. To verify onsite disinfection efficiency, samples collected following coagulation in the HydroSeparator were disinfected with three different concentrations of PAA in the laboratory. The removal of *Enterococcus* via laboratory disinfection was 2.4 log units when the same dose of PAA was applied as onsite disinfection. Overall, the removal of *Enterococcus spp* was seen as being comparable between laboratory and full-scale disinfection (Paper III).

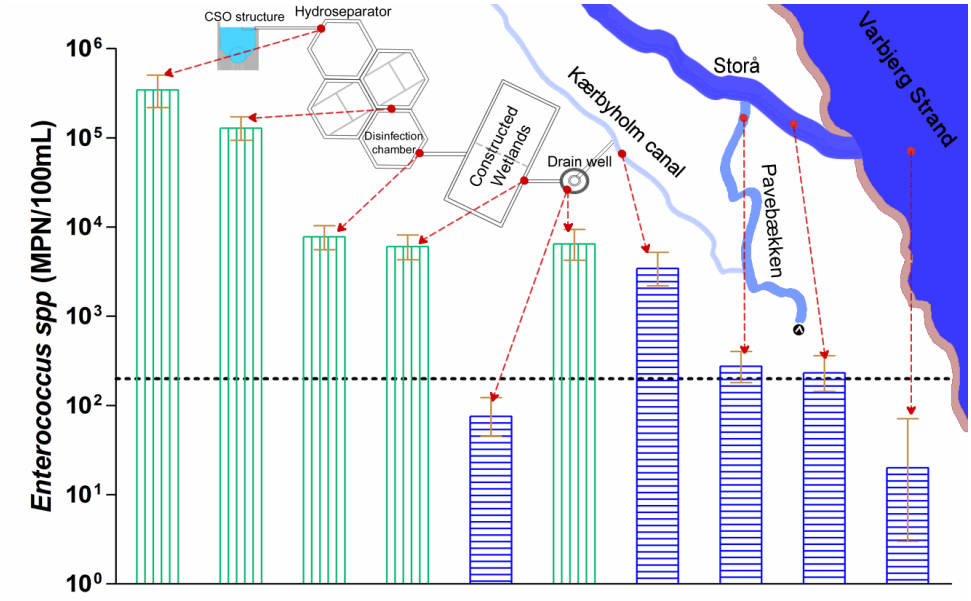

Figure 11: The number of *Enterococcus spp* from samples collected at different locations in the CSO structure and the recipient water. The blue bar with the horizontal pattern is the number of *Enterococcus spp* in the receiving water prior to the CSO event. T-bars indicate a 95% confidence interval (Paper III).

The microbial profile of *Enterococcus spp* was composed by measuring samples from different locations in the CSO structure and the receiving waters, before and after CSO events. Samples were collected for the microbial profile based on the flow of CSO water and retention time in the CSO structure. This was done so that the quality of CSO water from the inlet of the HydroSeparator matched when it reached the receiving water. Furthermore, samples from the drain well, the Kærbyholm canal, Pavebækken, Storå and Varbjerg Strand were collected prior to the CSO event, to obtain information on the pre-existence of *Enterococcus spp* (Figure 11). Initial *Enterococcus spp* concentration was $10^{5.5}$ MPN per 100 mL during the CSO event, but this reduced to $10^{3.7}$ MPN per 100 mL after the disinfection chamber, when treated in the HydroSeparator and disinfected in the disinfection chamber. The number of *Enterococcus spp* was

almost constant throughout the constructed wetland. In the first receiving surface water, namely the Kærbyholm canal, the concentration was almost the same as the effluent from the treatment system, albeit following dilution into the larger streams of Pavebækken and Storåen, the number of *Enterococcus spp* will decrease to around the bathing water criteria (Paper III).

Overall, a combined treatment of chemical coagulation, a flow through treatment in the HydroSeparator and disinfection by PAA successfully removed the suspended solids and a pollutant associated with SS and indicator bacteria, *Enterococcus spp*, which was regulated by the EU bathing water directive for safe recreational purpose.

3.3 Arctic WW treatment with PAA

PAA was used to disinfect arctic wastewater, which has a similar characteristic to CSO water. The main objective of this study was to investigate whether PAA can be used as a disinfectant to treat arctic wastewater. The Arctic has unique environmental and infrastructural conditions; therefore, construction of conventional wastewater treatment plants poses several challenges. First, a treatment facility cannot be constructed with open basins, due to the risk of water freezing during winter time, and even during periods without freezing, low average temperatures would result in significantly increased retention times and thus basin sizes – due to low biological and chemical treatment activity (Gunnarsdóttir et al., 2013). To overcome these problems, a quick treatment of wastewater by chemical coagulation followed by PAA disinfecting arctic domestic wastewater was performed. The overall goal was to achieve a satisfactory quality of wastewater for discharge, without a negative impact on the marine environment or human health (Paper V). The experiments were conducted in Denmark and Greenland on a batch scale. Experiments in Denmark were conducted to optimise the coagulation and disinfection methods for wastewater treatment in Greenland, and experiments in Greenland were conducted to study the feasibility of coagulation and disinfection.

The coagulant dose was optimised by employing a jar test, using different concentrations of PAX XL 100 in the wastewater collected from Lundtofte WWTP. A wastewater sample coagulated with 5 mg-Al/L was disinfected with 2, 6 and 12 mg/L PAA, and the degradation of PAA was observed for 60 minutes. Apparently high initial consumption was observed in all samples (Figure 12).

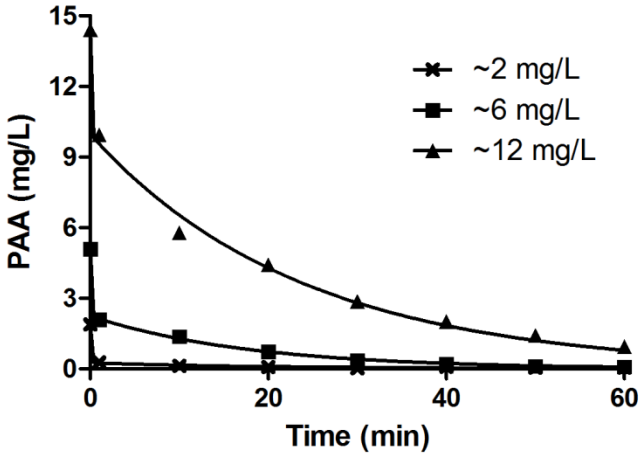

Figure 12: Concentration profile of different levels of PAA in the coagulated wastewater. The curve was fitted using modified first-order degradation kinetics and equation 5 (Paper V).

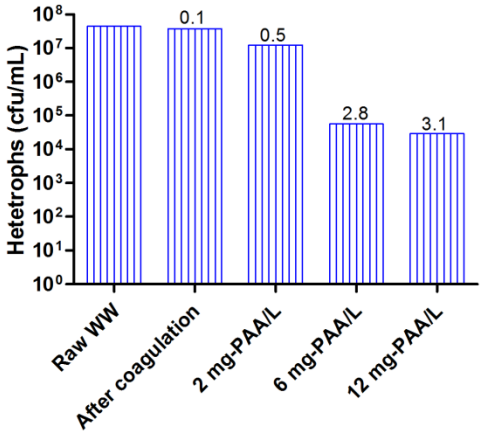

Figure 13: Removal of heterotrophic bacteria from raw wastewater treated with chemical coagulation and PAA disinfection. Numbers above the bars in the graphs represent the log10 removal of microorganisms after chemical coagulation and disinfection (Paper V).

Modified first-order degradation kinetics, as presented in equation 5, was used to fit the observed data. The fast degradation of PAA might be due to the oxidation of organics present in the wastewater. The number of heterotrophic bacteria was reduced from $10^{7.6}$ CFU/mL to $10^{4.8}$ CFU/mL and $10^{4.5}$ CFU/mL, respectively, after disinfection with 6 mg/L and 12 mg/L PAA over 60 minutes' contact time. However, due to the rapid consumption of 2 mg/L PAA, heterotrophic bacteria reduced by only $10^{7.1}$ CFU/mL from $10^{7.6}$ CFU/mL (Figure 13 & Paper V). The experiment on PAA disinfection in Greenland was not conducted, due to a temporary problem involving the delivery of chemicals to

Greenland. In summary, heterotrophic bacteria were reduced in wastewater by combining chemical coagulation followed by chemical disinfection by PAA.

4 Conclusion

As presented in this thesis, PFA and PAA showed their potential use for CSO disinfection by removing efficiently the indicator organisms *E. coli* and *Enterococcus spp*. PAA is suitable for treating CSO water with a long retention time, while PFA was efficient in reducing *E. coli* and *Enterococcus spp* at low doses and short contact times. The quick degradation of PFA made it suitable for disinfection in the CSO structure with a short retention time. Parameters that are necessary for CSO disinfection were investigated in laboratory simulated CSO water by applying different concentrations of disinfectants with different contact times. Disinfection mechanisms of PAA on *E. coli* were studied, and PAA without hydrogen peroxide showed similar disinfection efficiencies as commercial PAA. A rapid colorimetric assay for PAA and PFA quantification was developed, using ABTS and DPD colorimetric assays, which are necessary to control the disinfection process.

An ecotoxic evaluation of PFA, PAA, PAA without hydrogen peroxide, ClO_2 and their degradation products hydrogen peroxide and chlorite on organisms from different tropic levels in the aquatic ecosystem was carried out. Disinfectants were more toxic than their by-products, i.e. PFA was more toxic to *V.fischeri* and *D. magna*, and ClO_2 was more toxic to *P.subcapitata* compared to the other disinfectants studied. PNEC, EQS_{water} and MAC-$QS_{fw,eco}$ values of PFA, PAA, PAA without hydrogen peroxide, ClO_2, hydrogen peroxide and chlorite were derived for an indicative environmental risk assessment. Dilution factors of the disinfectants and their degradation products were calculated for the post-disinfection discharge of CSOs into receiving waters. PFA and ClO_2 needed maximum dilutions of 590 and 700 in relation to the receiving waters, to avoid the risk of toxic effects in the aquatic environment. However, due to the fast degradation of PFA and ClO_2 in water, the small dilution factors indicate that toxic effects will not occur after the initial dilution.

Both PAA and PFA were used for the full-scale disinfection of CSO water in the real CSO structure. PAA was effective in reducing *Enterococcus spp* when CSO water was pre-treated with a chemical coagulation with PAX XL 100. PFA reduced the numbers of *E. coli* and *Enterococcus spp* in the CSO water with short contact times and low dosages when applied to full-scale disinfection. The full-scale disinfection of both PAA and PFA was verified by applying a similar dose to the untreated CSO in the laboratory. Similarities in bacterial removal via full-scale and laboratory disinfection were observed.

This thesis has presented scientific knowledge on the chemical disinfection of CSO from laboratory to full-scale. A number of closure days can be reduced for harbour bathing areas allocated for recreational activities by disinfecting CSO before discharging into it. Moreover, environmental effects on the aquatic ecosystem were also considered when disinfected CSO was discharged into receiving waters.

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6 Papers

I **Chhetri R.K.**, Thornberg, D., Berner, B., Gramstad, R., Öjstedt, U., Sharma, A.K., Andersen H.R.: Chemical disinfection of combined sewer overflow waters using performic acid or peracetic acids. *The Science of Total Environment 2014, 490,* 1065-1072

II **Chhetri R.K.**, Flagstad, R., Munch, E.S., Hørning, C., Berner, J., Kolte-Olsen, A., Thornberg, D., Andersen H.R.: Full-scale evaluation of combined sewer overflows disinfection using performic acid in a sea-outfall pipe. *Chemical Engineering Journal 2015, 270,* 133-139

III **Chhetri R.K.**, Bonnerup, A., Andersen H.R.: Combined Sewer Overflow pre-treatment with chemical coagulation and a particle settler for improved peracetic acid disinfection. *Journal of Industrial and Engineering Chemistry 2016, 37*, 372-379

IV **Chhetri R.K.**, Baun, A., Andersen H.R.: Algal toxicity of the alternative disinfectants performic acid (PFA), peracetic acid (PAA), chlorine dioxide (ClO_2) and their by-products hydrogen peroxide (H_2O_2) and chlorite (ClO_2^-). *International Journal of Hygiene and Environmental Health 2017, 220*, 570-574

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VII **Chhetri R.K.**, Kaarsholm, K.M.S., Andersen H.R.: Colorimetric quantification of peroxycarboxylic acid and hydrogen peroxide for water disinfection. *Submitted*

VIII **Chhetri R.K.**, Di Gaetano, S., Turolla, A., Albrechtsen, H-J., Antonelli, A., Andersen H.R.: Study of disinfection efficiency of peracetic acid (PAA) on *Escherichia coli* after eliminating hydrogen peroxide from the commercial PAA mixture. *Manuscript*

IX **Chhetri R.K.**, Di Gaetano, S., Turolla, A., Antonelli, A., Andersen H.R.: Synergic effect of peracetic acid and hydrogen peroxide on *Escherichia coli* disinfection. *Manuscript*

X **Chhetri R.K.**, Di Gaetano, S., Turolla, A., Antonelli, A., Andersen H.R.: 10. Ecotoxicity evaluation of pure peracetic acid (PAA) after eliminating hydrogen peroxide from commercial PAA. *Manuscript*

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